IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/705,432	Confirmation No. 4884
Applicant	:	AUERBACH et al.	
Filed	:	10 November 2003	
TC/A.U.	:	1632	
Examiner	:	MONTANARI, David A.	
Docket No.	:	784	
Customer No.:		26693	

DECLARATION UNDER 37 CFR § 1.132

I, David FRENDEWEY, Ph.D., declare as follows:

1. I am a co-inventor of the invention described and claimed in the above-identified patent application. I presently hold the position of Senior Director, Targeted ES Cell Production at Regeneron Pharmaceuticals, Inc., the assignee of this patent application, located at 777 Old Saw Mill River Road, Tarrytown, New York 10591.

2. I have over 25 years of experience in the field of molecular biology. My experience includes, but is not limited to, developing improvements in methods for targeting specific loci in the genomes of mouse embryonic stem (ES) cells. A biographical sketch is attached as Exhibit 1.

3. I am aware that claims 17-32 have been rejected by the Examiner and that claims 25-28 are amended and now under examination. I have read the rejected claims and I have read the amended claims.

4. I understand that the Examiner has taken the position that the claimed invention is obvious in light of Rohozinski et al. (2002) Genesis, 32:1-7 (which I will call "Rohozinski"); Tsirigotis et al. (2001) BioTechniques 31:120-130 (which I will call "Tsirigotis"); and Ghazizadeh et al. (1998) J. Invest. Dermat. 111:492-496 (which I will call "Ghazizadeh"). I have read each of the references.

5. I have read the Examiner's Office Actions dated November 1, 2007, June 5, 2007, and August 28, 2007 and considered the Examiner's obviousness arguments.

6. Below I explain why the claimed invention would not have been obvious to a

person of skill in the art in light of the cited references, why the claimed invention would not have been obvious based on the knowledge of a person of skill in the art, and why the results of the claimed invention were unexpected and surprising to a person of skill in the art at the time the invention was made.

7. In my opinion, it is incorrect to assert that a person of ordinary skill in the art would select the ubiquitin promoter to solve the problem of low targeting frequency of a targeting construct having a PGK promoter because it would have been obvious to do so.

8. First, I disagree with the Examiner that there is any suggestion or understanding in the art that a ubiquitin promoter would express better at a specific chromosomal location than a PGK promoter and would thus be a better choice to use in a targeting vector than the PGK promoter. There is no motivation or suggestion in the art of which I am aware that would suggest a ubiquitin promoter would be any more active than a PGK promoter at the same chromosomal location where the PGK promoter has a low activity.

9. Second, I disagree with the Examiner's position that a person of ordinary skill would be motivated to select a ubiquitin promoter because a ubiquitin promoter has inherent properties known to those of ordinary skill that would recommend its use in targeting a specific chromosomal location that is poorly targeted using a PGK promoter. There is no indication in the art of which I am aware that would suggest an inherent property of the ubiquitin promoter that would result in better targeting at a specific chromosomal location where using a PGK promoter results in poor targeting.

10. Third, I disagree with the Examiner's position that the ubiquitin promoter would be an obvious choice to increase targeting efficiency because the ubiquitin promoter is able to drive expression in a large variety of cells and in a large variety of tissues (a feature shared by a great many promoters of, for example, "housekeeping genes"). The ability of a promoter to drive expression in many tissues or many cell types is unrelated to its ability to drive expression at a specific chromosomal location.

11. I disagree with the Examiner's assertion that any combination of Rohozinski, Tsirigotis, and Ghazizadeh, taken alone or together with the understanding of a person of ordinary skill in the art, would make it obvious to choose a ubiquitin promoter in a targeting vector designed to target a specific chromosomal location where the PGK promoter is unable to function or results in poor targeting efficiency.

12. Tsirigotis rationally promotes using a ubiquitin promoter in a transgenic construct for random insertion into a genome because it is known to be one of the best promoters for the tissue-independent expression of transgenes in mice. This property has no bearing on whether the promoter is able to drive expression at a specific, targeted chromosomal location, for example, at a specific location where the PGK promoter is unable to drive expression or drives expression only poorly and does not instruct as to how the promoter affects gene targeting efficiency or success. When considering the ability to drive expression at a specific chromosomal location, such as one where the PGK promoter is unable to drive expression, a person of ordinary skill in the art would understand that Tsirigotis provides no guidance in this regard.

13. Rohozinski shows specialized methods for homologous recombination on the Y chromosome using an insertional targeting construct, as opposed to a standard replacement construct, having a pol II promoter driving neo^r expression. Rohozinski tells us that the high rates of homologous recombination on the Y chromosome that they obtained were made possible by the design of their insertional targeting vectors and the use of spermidine in the electroporation buffer. They make no mention of the effects on targeting efficiency and success, positive or negative, of promoters used to expression the neo^r gene. A person of ordinary skill seeking to improve targeting efficiency over that observed in a construct having a PGK promoter would not look to Rohozinski, because Rohozinski tells us nothing about how the promoter of the drug selection gene affects gene targeting efficiency or success.

14. Ghazizadeh would provide no insight to a person of ordinary skill, because like Tsirigotis, Ghazizadeh is concerned with random integration into a genome, not integration at a specific chromosomal location. There is no rational basis to combine Ghazizadeh with either or both of Tsirigotis and Rohozinski, taking into account what is known by a person of ordinary skill in the art, because there is no nexus between any of these references and the concept of increasing targeting efficiency by using a ubiquitin promoter at a specific chromosomal location where a PGK promoter provides unsatisfactory targeting. Ghazizadeh does not mention targeting to a specific gene or locus and neither instructs on the general effects of the promoters used to express the neo^r gene nor on the ubiquitin promoter, specifically.

15. In summary, a person of ordinary skill would find no guidance in the cited

references or in the knowledge in the art that would recommend using a ubiquitin promoter at a chromosomal location that a PGK promoter is unsatisfactory. Similarly, there is no guidance in the references or within the knowledge of a person of skill in the art that would suggest that a ubiquitin promoter driving a selection marker in a targeting construct would perform any better at a specific chromosomal location than a PGK promoter.

16. Because there are no useful guidelines for selecting which of the commonly used promoters known in the art would be active at a specific chromosomal location where a PGK promoter is inactive, a person of ordinary skill would most likely conduct an empirical study of several promoters to ascertain which promoters would exhibit better expression than the PGK promoter at the specific locus in question.

17. Such an empirical study would be carried out, for example, by examining the level of expression of mRNA of a marker gene operably linked to each promoter at the specific chromosomal location, and comparing expression of the promoters to expression driven by PGK at the same chromosomal location. A person of ordinary skill would reasonably expect that a promoter that results in a higher level of mRNA expressed from the marker gene operably linked to it would be the better promoter and thus should result in a more desirable targeting efficiency. Essentially the same experiment was conducted at my direction, as described below.

18. Exhibit 2 shows the results of an experiment where individual ES cells were electroporated with targeting constructs directing a neo^r selection marker driven by either a PGK promoter (clone 216A-D10) or a ubiquitin promoter (clones 531B-F10, 531B-H4, and 531B-A6), as described in the patent application at Example 1, and relative expression of the neo^r gene was determined. The targeting vectors were all directed to the same chromosomal location (the mouse Fbx25 locus), and proper targeting resulted in an Fbx25 deletion and replacement with a lacZ gene, as well as neo^r under the control of either a PGK or ubiquitin promoter. Expression as measured by quantitiative PCR of mRNA to neo^r, normalized to FGFR2 mRNA (open bars) and normalized to GADPH mRNA (closed bars) indicated that expression of the neo^r gene at the Fbx25 locus was roughly the same for the three clones having the ubiquitin promoter (68/61, 81/81, and 59/73), and expression of the neo^r gene from the same locus driven by the PGK promoter was slightly higher (105/75). Based on these results, a person of ordinary skill would conclude that the PGK promoter and the ubiquitin promoter each express effectively at this specific

chromosomal location, and that the PGK promoter may be slightly stronger. A person of ordinary skill would rationally conclude from this experiment that there is no advantage to replacing the PGK promoter with a ubiquitin promoter to target this specific chromosomal location.

19. Exhibit 3 shows the results of a quantitative PCR to quantitate the level of neo^r expression at a specific chromosomal locus driven by a PGK promoter (clone AD10) and a ubiquitin promoter (clones BF10, BH4, and BA6), normalized to FGFR1 and GAPDH mRNAs. The lower the delta Ct value, the higher the expression. Exhibit 3 shows that PGK promoter-driven and ubiquitin promoter-driven expression at the specific chromosomal locus was about the same (average delta Ct of 2 for the PGK promoter, average delta Ct of slightly over 2 for the ubiquitin promoter). When the average delta Ct for PGK-driven neo^r expression normalized to FGFR1 is compared to the average delta Ct for UbC-driven neor expression normalized to FGFR1 , relative expression (PGK:UbC) is 1.53. When the normalization is with respect to GADPH, relative expression (PGK:UbC) is 1.05. Thus, the PGK promoter is about the same or better at driving expression from the same specific chromosomal location.

20. Thus, the empirical evidence would lead a person of ordinary skill to conclude that the ubiquitin promoter would afford no advantage at the specific chromosomal locus where PGK poorly drives expression, because the empirical evidence indicates that the ability of each promoter to drive expression is not significantly different and, if anything, PGK is a bit better. Surprisingly, however, we have found that substituting the ubiquitin promoter for the PGK promoter at the same locus (the locus of the above-described experiments) results in a higher targeting efficiency. Moreover, we have observed that the ubiquitin promoter is able to rescue targeting at a number of loci that cannot be targeted using a marker gene driven by the PGK promoter.

21. Further, we have discovered that loci that are poorly targeted by use of the PGK promoter to express a selectable marker frequently have multiple inserts of the construct comprising the PGK promoter, whereas constructs having a ubiquitin promoter to express a selectable marker that rescue the poor targeting with the PGK promoter generally produce clones with a single copy insert. We believe that this phenomenon is in part responsible for the unexpected ability of the ubiquitin promoter to rescue targeting at loci that cannot be effectively targeted by the PGK promoter. There is no guidance in the

art that would suggest that ubiquitin would rescue targeting at a locus over PGK due to a difference in copy number of the insertion.

22. It is unexpected and surprising that using a UbC promoter to drive a selectable marker would rescue targeting at a specific locus over a PGK promoter driving the same selectable marker. Our discovery that there is an inverse correlation between insert copy number and targeting frequency for the PGK and ubiquitin promoters is not disclosed or suggested in the art. A person of ordinary skill would rationally assume that multi-copy inserts would result in more marker activity and thus a better targeting frequency. We found the reverse to be true. Constructs that include the UbC promoter driving selectable marker expression more frequently produce single copy insertions and higher targeting efficiencies than those in which selectable marker expression is driven from the PGK promoter.

23. For the reasons stated above, it is my opinion that the claimed invention would not be obvious to a person of ordinary skill in the art at the time the application was filed.

24. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

01-April-2008
Date

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EXHIBIT 1 TO FRENDEWEY DECLARATION DATED 01 APRIL 2008

BIOGRAPHICAL SKETCH

NAME **FRENDEWEY**, David	POSITION TITLE **Director Targeted ES Cell Production**
eRA COMMONS USER NAME N/A	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Michigan Technological University, Houghton, MI	B.S.	May 1976	Biology
University of Wyoming, Laramie, WY	Ph.D.	Aug. 1980	Biochemistry
Yale University, New Haven, CT	Postdoctoral	1980–1983	Biochemistry
German Cancer Res. Center, Heidelberg,	Postdoctoral	1984–1986	Molecular Biology

A. Employment.

1. **Senior Staff Investigator**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1987–1991
2. **Assoc. Professor**, Dept. of Microbiology, New York University School of Medicine, New York, NY, 1991–1997
3. **Staff Scientist**, Biomolecular Sciences, HTS Assay Development, Regeneron Pharmaceuticals, Inc., 1998–2001
4. **Associate Director**, Targeted ES Cell Production, VelociGene Program, Regeneron Pharmaceuticals, Inc., 2002–2004
5. **Director**, Targeted ES Cell Production, VelociGene Program, Regeneron Pharmaceuticals, Inc., 2005

B. Selected peer-reviewed publications (in chronological order).

1. **Frendewey, D.A**. and Kaiser, II. 1979. Effects of 5-fluorouracil on base modification in Escherichia coli tRNA. Biochemistry *18*:3179-3185.
2. **Frendewey, D.A.**, D.M. Kladianos, V.G. Moore and Kaiser, II. 1982. Loss of tRNA 5-methyluridine methyltransferase and pseudouridine synthetase activities in 5-fluorouracil and 1-(tetrahydro-2-furanyl)-5-fluorouracil (ftorafur)-treated Escherichia coli. Biochim Biophys Acta *697*:31-40.
3. Hottinger, H., B. Stadelmann, D. Pearson, **D. Frendewey**, J. Kohli and D. Soll. 1984. The Schizosaccharomyces pombe sup3-i suppressor recognizes ochre, but not amber codons in vitro and in vivo. Embo J *3*:423-428.
4. **Frendewey, D.**, T. Dingermann, L. Cooley and D. Soll. 1985. Processing of precursor tRNAs in Drosophila. Processing of the 3' end involves an endonucleolytic cleavage and occurs after 5' end maturation. J Biol Chem *260*:449-454.
5. **Frendewey, D.** and W. Keller. 1985. Stepwise assembly of a pre-mRNA splicing complex requires U-snRNPs and specific intron sequences. Cell *42*:355-367.
6. Kaiser, II, D.M. Kladianos and **D.A. Frendewey**. 1985. Specific incorporation of 5-fluorocytidine into Escherichia coli RNA. Biochim Biophys Acta *825*:12-20.
7. Brown, J.W., G. Feix and **D. Frendewey**. 1986. Accurate in vitro splicing of two pre-mRNA plant introns in a HeLa cell nuclear extract. EMBO J *5*:2749-2758.
8. Krupp, G., B. Cherayil, **D. Frendewey**, S. Nishikawa and D. Soll. 1986. Two RNA species co-purify with RNase P from the fission yeast Schizosaccharomyces pombe. Embo J

5:1697-1703.

9. Willis, I., **D. Frendewey**, M. Nichols, A. Hottinger-Werlen, J. Schaack and D. Soll. 1986. A single base change in the intron of a serine tRNA affects the rate of RNase P cleavage in vitro and suppressor activity in vivo in Saccharomyces cerevisiae. J Biol Chem *261*:5878-5885.

10. Christofori, G., **D. Frendewey** and W. Keller. 1987. Two spliceosomes can form simultaneously and independently on synthetic double-intron messenger RNA precursors. Embo J *6*:1747-1755.

11. **Frendewey, D.**, A. Kramer and W. Keller. 1987. Different small nuclear ribonucleoprotein particles are involved in different steps of splicing complex formation. Cold Spring Harb Symp Quant Biol *52*:287-298.

12. Erster, S.H., L.A. Finn, **D.A. Frendewey** and D.M. Helfman. 1988. Use of RNase H and primer extension to analyze RNA splicing. Nucleic Acids Res *16*:5999-6014.

13. Potashkin, J. and **D. Frendewey**. 1989. Splicing of the U6 RNA precursor is impaired in fission yeast pre-mRNA splicing mutants. Nucleic Acids Res *17*:7821-7831.

14. Potashkin, J., R. Li and **D. Frendewey**. 1989. Pre-mRNA splicing mutants of Schizosaccharomyces pombe. Embo J *8*:551-559.

15. **Frendewey, D.**, I. Barta, M. Gillespie and J. Potashkin. 1990. Schizosaccharomyces U6 genes have a sequence within their introns that matches the B box consensus of tRNA internal promoters. Nucleic Acids Res *18*:2025-2032.

16. Potashkin, J. and **D. Frendewey**. 1990. A mutation in a single gene of Schizosaccharomyces pombe affects the expression of several snRNAs and causes defects in RNA processing. Embo J *9*:525-534.

17. Rotondo, G., M. Gillespie and **D. Frendewey**. 1995. Rescue of the fission yeast snRNA synthesis mutant snm1 by overexpression of the double-strand-specific Pac1 ribonuclease. Mol Gen Genet *247*:698-708.

18. Lundgren, K., S. Allan, S. Urushiyama, T. Tani, Y. Ohshima, **D. Frendewey** and D. Beach. 1996. A connection between pre-mRNA splicing and the cell cycle in fission yeast: cdc28+ is allelic with prp8+ and encodes an RNA-dependent ATPase/helicase. Mol Biol Cell *7*:1083-1094.

19. Rotondo, G. and **D. Frendewey**. 1996. Purification and characterization of the Pac1 ribonuclease of Schizosaccharomyces pombe. Nucleic Acids Res *24*:2377-2386.

20. Rotondo, G., J.Y. Huang and **D. Frendewey**. 1997. Substrate structure requirements of the Pac1 ribonuclease from Schizosaccharmyces pombe. Rna *3*:1182-1193.

21. Potashkin, J., D. Kim, M. Fons, T. Humphrey and **D. Frendewey**. 1998. Cell-division-cycle defects associated with fission yeast pre-mRNA splicing mutants. Curr Genet *34*:153-163.

22. McDonald, W.H., R. Ohi, N. Smelkova, **D. Frendewey** and K.L. Gould. 1999. Myb-related fission yeast cdc5p is a component of a 40S snRNP-containing complex and is essential for pre-mRNA splicing. Mol Cell Biol *19*:5352-5362.

23. Zhou, D., **D. Frendewey** and S.M. Lobo Ruppert. 1999. Pac1p, an RNase III homolog, is required for formation of the 3' end of U2 snRNA in Schizosaccharomyces pombe. Rna *5*:1083-1098.

24. Provost, P., D. Dishart, J. Doucet, **D. Frendewey**, B. Samuelsson and O. Radmark. 2002. Ribonuclease activity and RNA binding of recombinant human Dicer. Embo J *21*:5864-5874.

25. Ivakine, E., K. Spasov, **D. Frendewey** and R.N. Nazar. 2003. Functional significance of intermediate cleavages in the 3'ETS of the pre-rRNA from Schizosaccharomyces pombe. Nucleic Acids Res *31*:7110-7116.

26. Valenzuela, D.M., A.J. Murphy, **D. Frendewey**, N.W. Gale, A.N. Economides, W. Auerbach, W.T. Poueymirou, N.C. Adams, et al. 2003. High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol *21*:652-659.

Book Chapters

1. Steitz, J.A., Black, D.L., Gerke, V., Parker, K.A., Krämer, A., **Frendewey, D.** and Keller, W. 1988. Functions of the abundant U-snRNPs in The structure and function of small nuclear ribonucleoprotein particles, p. 115–154. *In* M.L. Birnstiel (Ed.),Springer, Berlin.
2. Rotondo, G. and **Frendewey, D.** 2001. The Pac1 Ribonuclease of Schizosaccharomyces pombe., p. 168–193. *In Methods Enzymol.*

EXHIBIT 2 TO FRENDEWEY DECLARATION DATED 01 APRIL 2008

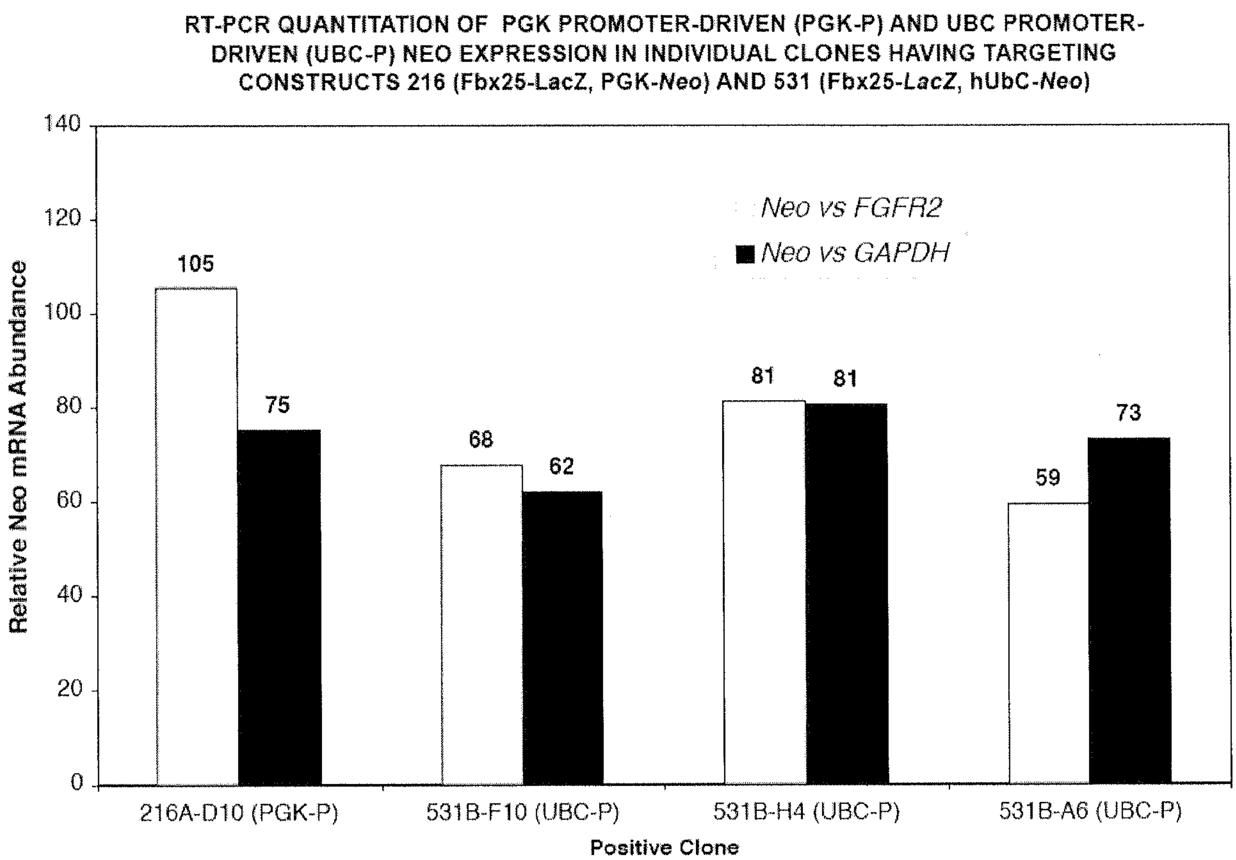

EXHIBIT 3 TO FRENDEWEY DECLARATION DATED 01 APRIL 2008